DISCUSSION

Anabas testudineus became excited after exposure to different concentrations of latex and their surfacing frequencies increased for a few minutes. The colour of the body became pale after about 6hrs. The observed behavioral changes in LC50 and subsequent higher doses, such as erratic jumping movements, changes in opercular movements rate, irregular swimming activity of the body, hyper and hypo activity, increase in surfacing activity, loss in equilibrium, spiraling, jerky movements, vertical movements spreading of excess mucus all over the surface of the body lead ultimately death occurred as the result of exposure to pesticide. These findings are in conformity with the earlier findings of Sabita borah and Yadav (1996); Pravakar et al., (1993); Sadhu (1993); Santha Kumar (1998); Prasanth et al., (2005); Mahajan and Patole (2003); Patole and Mahajan, (2006). Duration of latent period depend on the nature and concentrations of toxicant used and fish species (with other conditions remaining uniform). But in general, organic pesticides have a short latent period, thus the symptoms of poisoning start when the fishes are exposed. Changes in ventilation rate and surfacing frequencies are the general symptoms noticed in the fish after exposure to the pesticide and these activities help the fish to avoid the contact with poison and fight against the stress (Chowdhary et al., 1981; Roy and Munshi, 1987).

Fishes that have water-breathing and air-breathing organs are bimodal regarding breathing. Bimodal species are noted for their resistance to environmental stress and aquatic hypoxia (Dehadrai and Tripathi, 1976). The LC50 Value differs from species to species for the same pesticide and different pesticides due to the mode of their action on fish (King, 1962). The purified principle of Calotropis gigantea, 0.02-0.04 G when injected subcutaneously kills rabbit in 30 minutes, guinea pig in 15 minutes, in pigeon there results vomiting, in frogs causes systolic arrest with in 6 minutes (Nadkarni, 1991). 96 hr LC50 value for bio pesticide of Calotropis gigantea was found to be 10ml/5 liters (Bharathi, 2005), and latex was 0.1ml/5litres (Josaphene Paulina, 2003; Preethakumari, 2004) in the Anabas testudineus. Air
breathing fish have a higher LC$_{50}$ value compared to water-breathing fish (Dutta et al., 1992a). In the presence of toxin, these fishes reduce the opercular movement rate but increase the surfacing frequency leading to more air breathing. The observed mucus accumulation on the gills and skin of the fishes exposed to pesticides were probably due to toxic effects of pesticides, because respiratory epithelium might be the main target site of toxicity during the period of experiment. The mucus may be an adaptive response perhaps providing additional protection against corrosive nature of pesticides. This agrees with the earlier findings of Sadhu (1993), Pravakar et al., (1993) and pesticides of plant origin (Patole and Mahajan, 2004). The increased secretion of mucous by the skin made by body slipperiness of quick movement in the test solution and avoids the absorption of the toxicant by the general body surface (Sabita Borah and Yadav, 1995). Ichthyo toxins like saponins, cardiac glycosides, alkaloids, isoflavonoids, tannins, cynogenic compounds proved highly ichthyo toxic and these chemicals lower surface tension of water interfering respiration and central nervous system (acetylcholine inhibitors). Death of the fish may be due to the acetylcholine inhibition and the respiratory failure. It was noted that 90% of inhibition of Acetylcholinesterase resulted in death of the test fish (Sinha and Datta Munshi, 1996; Singh and Singh, 2005). All the organophosphate insecticides also act as a nerve poisons by blocking synaptic transmission and the cholinergic parts of a nerve (O'Brien et al., 1974). This results in the accumulation of Acetylcholine as synapses that produce severe physiological disturbances leading to titanic paralysis, respiratory failure via respiratory centre and death.

**BEHAVIOURAL TOXICITY**

**Optomotor behavioural Changes**

The optomotor response is essential for behaviour such as searching for food, orientation towards food, odor, location of a mate, escaping from a predator and avoidance of an adverse situation. Two major behavioral changes observed were “hypoactive” and “lethargy” exposed to different sublethal concentrations of latex,
latex plus supplements and plant extract. In *Anabas testudineus* hypo activity and lethargy was seen at the higher concentrations TU2(0.01ml/5liters) and TU3(0.02ml/5liters) of latex, TU2(1ml/5liters); TU3(2ml/5liters) of plant extract treated fish. The lethargic condition that results due to pesticide exposure would affect fish in several ways. The fish that became lethargic would fall easy prey to predators. Feeding and food capture will be hampered by lethargy and loss of orientation caused by the action of the pollutants. Fish living in streams may not be able to maintain their position and may be swept downstream. *Sharma et al., (1983)* reported erratic swimming movements followed by lethargy in *Clarias batrachus* exposed to 0.25 to 2 mg /l Malathion. Similar hypoactive and lethargic conditions were observed in fish *Labeo rohita* and *Anabas testudineus* exposed to malathion, *Dutta et al.,1992b,1994,* *Anabas testudineus* exposed to monocrotophos(*Santha kumar,1998*), *Anabas testudineus* exposed to latex of *Calotropis gigantea* (*Josephine paulina,2003;Preetha kumari,2004*). *Anabas testudineus* exposed to biopesticide prepared from *Calotropis gigantea* (*Bharathi, 2005*), *Cirrihnus mrigala* exposed to cypermethrin (*Prasanth et al., 2005*). From the present studies it appeared that the deviation from normal activity is pronounced, however, certain amount of recovery could be seen in latex plus supplements treated fish, enabling to withstand stress in toxic environment.

**Impact on Surfacing behaviour, Distance traveled and Opercular movement**

In the present study, the fish *Anabas testudineus* showed a continuous decline in the opercular movements and increase in surfacing behaviour when exposed to sublethal exposure of latex and plant extract. This coincides with the work done by *Anbu and Ramaswamy (1991)* in *Channa straiatus* exposed to sevin, *Zayapragassarazan (1993)* in *Anabas testudineus* exposed to lindane and *Sajitha Bhaskar (1934)* in *Anabas testudineus* exposed to endosulfan, *Anabas testudineus* exposed to monocrotophos (*Santha Kumar,1998*), *Anabas testudineus* exposed to latex of *Calotropis gigantea* (*Josephine Paulina,2003*) *Anabas testudineus* exposed to latex
of *Calotropis gigantean* (Preetha Kumari, 2004) *Anabas testudineus* exposed to biopesticide prepared from *Calotropis gigantea* (Bharathi, 2005). Changes in ventilation rate and surfacing frequencies are the several symptoms noticed in the fish after exposure to the pesticide and these activities help the fish to avoid the contact with poison and fight against the stress (Chowdhary et al., 1981; Roy and Munshi, 1987). *Anabas testudineus* have water breathing and air breathing organs (bimodal regarding breathing). Bimodal species are noted for their resistance to environmental stress and aquatic hypoxia (Dehadrai and Tripathi, 1976). These are because, air breathing supplements the oxygen requirements in these fishes. Further, they have reduced aquatic respiratory surfaces with a thicker barrier providing a lesser toxicant. It is possible that air breathing renders fish more resistance to toxicants by permitting the reduction of gill ventilation, thereby reducing contact with toxicant at a major site of uptake (Anbu and Ramaswamy, 1991). In these fishes opercular movement rate decreased, but surfacing frequency increased leading to more air breathing. Bimodal fishes respond to toxicants by reducing the proportion of oxygen uptake via gills (Kulakkatolickal and Karmer, 1988). The capacity to reduce the uptake of noxious substances should be added to the list of other possible advantages of bimodal respiration in fishes. Bull and Mc Inerney (1974) reported that many juveniles of Coho Solmon were unable to maintain position and where swept downstream after being exposed to sumithion, an organophosphorus insecticide, in a stream aquarium. The results of the present study show that exposure of fish to latex, plant extract in the aquatic environment may not cause immediate death, but it certainly can cause some undesirable behavioural changes. These undesirable behavioral changes may lead to a reduction in the number of fish population that will result in a disturbance in the aquatic ecosystem. However, certain amount of recovery could be seen in latex plus supplements treated fish, enabling to withstand stress in toxic environment and the nutrients, that provide strength and support to fish.
TOXICITY IMPACT ON HEMATOLOGICAL PARAMETERS

Haemolysis of red blood cells provides simple and rapid way of studying the effect of pollutants on biological membranes (Harington et al., 1971) numerous investigations have considered membrane model a measure of pollutant’s cytotoxicity (Allison et al., 1966). The red blood cell membrane haemolysis has proved to be a simple and rapid way of attempting to find the possible correlation between toxicity and haemolytic activity (Macnab and Harington, 1967). In the present study a clear trend was observed linking latex and plant extract concentration with membrane damage. The impact was the most severe on fishes exposed to the highest of the three sublethal concentrations of latex, plant extract of Calotropis gigantea. In this concentration severe lysis was also observed. The next lower concentration of latex and in the plant extract exposed fishes caused lysis of a less severe magnitude. The present experiments revealed that Haemoglobin content, RBC, Ht and MCHC were significantly lesser in the latex, plant extract exposed fishes. The most pronounced depression in RBC count, Ht, MCHC and Hb content were in the fishes exposed to the highest of three sublethal concentrations and decrease was progressively lesser in the lesser concentrations. Oxygen combining capacity of blood is direct function of its Hb content. The trends observed with this parameter were exactly parallel to the trends observed for Hb content. The similar decrease in Hb, RBC count, Ht and MCHC was observed in rats exposed to monocrotophos and its analogues (Siddiqui et al., 1991) and in Anabas testudineus exposed to monocrotophos (Santha Kumar, 1998), Anabas testudineus exposed to biopesticide prepared from Calotropis gigantea (Bharathi, 2005). Decrease in Hb content, RBC count, Ht and MCHC was also observed in fishes exposed to other pesticides: fenproparthrin (Ahmad Figar et al., 1995); paraquant (Ibrahim et al., 1995); malathion (Ikhair-ud-Din et al., 1996) praquant (Ibrahim et al., 1995); Methyl parathion (Nath Rabindra and Banerjee, 1996). Haematological studies disclose possible reaction of blood and blood forming organs to the pesticide treatment (Siddiqui et al., 1987; Srinivasan and Radha Krishnamurthy, 1983). Janardhan and Sisodia (1990) reported
decrease in the Hb concentration, erythrocyte counts in rats exposed to monocrotophos. In the present study total RBC, oxygen combining capacity of blood and Hb content showed a decreasing trend with increasing exposure time. The reduction in RBC number may be due to microcytic or normocytic anaemia (Tuschiya, 1973). Janardhan and Sisodia (1990) observed decrease in haemoglobin concentration with concomitant increase in bilirubin concentration indicates haemolytic condition that could be the result of direct toxic effect of monocrotophos on erythrocytes. In most vertebrates, including, fish erythropoietic activity is regulated by erythropoietin produced in the kidney (Gordon et al., 1967). Erythropoietin, besides promoting erythropoiesis by indicating; hemopoietic stem cells to differentiate into erythroblasts, which forms RBC, also makes pyridoxal phosphate active in developing RBC’S inducing Hb synthesis. Hypoxia constitutes the fundamental stimulus for erythropoiesis with the kidney as the probable sensing organ for low blood oxygen tensions. (Jacobsen and Krantz, 1968). Latex, plant extract, of Calotropis gigantea like several other organophosphates, impairs neuromuscular transmission through acetylcholineesterase inhibition (Preetha Kumari, 2004) resulting in a reduction or cessation of respiratory movements as observed in behavioral studies and a decrease in oxygen uptake. In the present studies, Anabas testudineus showed a decrease in RBC counts suggesting a decrease in erythropoietic activity. A structurally intact and normally functioning kidney is essential for erythropoietin revealed progressive dystrophic changes in the kidney tubules of Anabas testudineus exposed to the three sublethal concentrations of monocrotophos (Santha Kumar, 1998). Kidney damage usually causes decrease in erythropoietin levels which in turn decrease RBC production and Hb synthesis even under pesticide induced stress condition. The significant decrease in Ht value in fish is correlated with a decrease in RBC, which might be due to its effect on blood forming organs (Srinivasan and Radhakrishnamurthy, 1983). The slight increase and decrease observed in MCHC and MCH values cannot be attributed to cells shrinking or swelling but rather to a proportional decrease in Red blood cells and
haemoglobin concentration. The significant increase in MCV result of swelling may be due to beta adrenergic stimulation caused by exposed stress condition experienced by the test organisms to latex and plant extract of Calotropis gigantea (Butler et al., 1978 and Santha Kumar, 1998). An increase in MCV value may be considered as RBC destruction leading to anaemia (Johansson-Sjoback and Larson, 1978). The decreased MCHC also points to the fact that cells swelling occurred. These findings in Anabas testudineus exposed to latex and plant extract of Calotropis gigantea are in partial agreement with the results of other researches (Dalela et al., 1981; Mishra and Srivastava, 1983; El-Doimary 1987; Gill et al., 1991a, b; Dutta et al., 1992c, Santha kumar, 1998; Josphene paulina, 2003). The leukocytes or white blood corpuscles are colorless due to the absence of haemoglobin. They play a crucial role in defending the animal against the invading toxins. They contribute to the immune response by destroying harmful bacteria and inactivating harmful foreign materials in the tissues and blood. The results of our experiment reveals that exposure to latex and plant extract of Calotropis gigantea caused increase in WBC count. In all the cases the increase in the WBC count was the highest in the higher of the sub-lethal concentration of latex and was progressively lower in the lower concentrations. The increase in WBC count may be attributed to the response of the fish to latex and plant extract of Calotropis gigantea, where latex and plant extract may act as an antigen. The significant increase in the total leukocyte count corroborates the earlier work on fishes (Ahamad Figar et al., 1995; Ibrahim et al., 1995; Ikhair-Ud-Din et al., 1996; Nath Rabindra and Banerjee, 1996). WBC is inextricably involved in the regulation of immunological function and prolonged exposure of Anabas testudineus to latex and plant extract may inflict immunological deficiency. The WBC count decreased slightly in the fishes exposed to three sublethal concentrations of latex plus supplements treated fish however, certain amount of recovery could be seen protecting from anaemic condition, enhancing immune power enabling to withstand stress in toxic environment. The present findings are in agreement with Santha
TOXICITY IMPACT ON BIOCHEMICAL PARAMETERS

Protein Levels

Proteins play a vital role in the biological functions and are, hence aptly called the building blocks for cellular components. In fish, proteins are the primary energy source and are involved in regulating physiological and metabolic processes in the body through hormones, enzymes etc., They play a vital role as energy precursors in fishes exposed to stress conditions (Jones Nelson and Sunil Kumar, 1996; Anitha Kumari and Sree Ram Kumar, 1996; Ramalingam, 1980). The Present experiment indicates that sub-lethal concentrations of latex and plant extract of Calotropis gigantea have a cognizable impact on the brain, liver, gill protein levels of Anabas testudineus. Protein levels were found to decrease and the significant decrease was observed in almost all organs. The decrease in protein levels was in the order of liver, gill and brain. Latex treated fishes were more affected than plant extract treated fishes. The absence or slight changes in alterations in the early periods of exposure supports the concept of Fry (1971) that fishes tend to resist stress or a specific period but may eventually succumb as result of their inability, to endure further. Umminger (1970) attributes this to the fact that proteins are reserves of energy, which are used up gradually during the periods of stress. Rapid loss of brain proteins during pesticide toxicity was reported (Richardson, 1981). The decrease in total tissue proteins indicates their metabolic utilization (Swamy et al., 1992b). The sub-lethal concentrations of latex and plant extract of Calotropis gigantea reduced the brain protein content (Josephine Paulina, 2003; Preetha Kumari, 2004) and Bharathi (2005) in Anabas testudineus with biopesticide of Calotropis gigantea. Santha Kumar (1998) with monocrotophos in Anabas testudineus gill, liver and brain. Swamy et al., (1992b) reported decrease in protein content in rat brain, Tilapia mossambica (Joshi and Desai, 1983) exposed to monocrotophos. Patil et al., (1990) reported in
estuarine edible Mudskipper *Boleophthalmus dussumieri* that the muscle protein and liver protein were reduced on exposure to sublethal concentrations of monocrotophos as observed in the present study. This decline in protein synthesizing capacity of the liver receives support for significant decline in the protein content of gills, liver and brain. The loss of protein under latex and plant extract stress noticed in the present study may be attributed to utilization of amino acids in the various catabolic reactions. The amount of protein becomes reduced when the concentration and the period of exposure increases. Lesion may result in the loss of protein and lead to the death of fish. *Swamy et al., (1992b)* reported that increase in the acid, neutral and alkaline proteases in rat brain and the increase in the activity of proteases correlated with the decrease of total protein. *Zayaprgassarazan and Anandan (1996)* reported significant decline in protein level and decrease in protein patterns in gills, liver, and brain of *Anabas testudineus* exposed to lindane. *Gray and Meckenzie (1970)* have stated that protein levels in fishes are not influenced by inherent factors like sex, stage of maturation and geographical location. Therefore, the alterations in the protein levels, which are noticed in the present study, may be due to the influence of exogenous factor like toxic environment as has been suggested by *Castell et al., (1970)*. *Nammalwar (1984), Anita Kumari and Sree Ram Kumar (1996)* reported changes in protein fraction in the gill, liver, and brain in mullets exposed to DDT and BHC and in pollution stressed fish as observed in brain, liver and gills in the present study. The altered mobility and low content of proteins in muscles reflect a change in the rate of synthesis and degradation of protein, lowered working capacity under the impact of stress. Fractions from the liver of the pesticide treated fish also displayed significant changes. These changes may be due to the accumulation of pollutants in the liver leading to an alteration in its function indicating the vulnerability of the organ. In the tissues, Lysosomal (acidic) proteases found to have role in protein degradation *(Marzella et al., 1981)*. It was reported that impaired energy supply leads to the break down of tissue proteins then susceptible to the action of tissue proteolytic enzymes and leading to their consequent degradation by proteases.
Depletion of tissue proteins has been reported by several investigators in fishes exposed to pesticides: Quinalphos (Anusha Amali et al., 1996); ekalux (Jones Nelson and Sunil Kumar, 1996); aldrin (Singh and Srivastava, 1995); nuvan (Tazeem Arasta, 1996; Anuradha, 1993); aldrin and propoxur (Singh et al., 1996) reported significant decrease in protein levels in *Tilapia mossambica* exposed to monocrotophos. Significant decline in the nucleic acid content has been reported earlier in the fish, *Tilapia mossambica* subjected to monocrotophos (Joshi and Desai, 1988). The lower protein synthesis indicating lower metabolism in these fishes. In the present study decrease in protein could be attributed to the enhanced activities of proteases, lower protein synthesis at the transcriptional level of impaired incorporation of amino acids into polypeptide chain. In latex plus supplements treated fishes, the difference in values were less significant. However, certain amount of recovery could be seen. It was interesting to note that, there was a tendency of these values to come nearer to control values of selected tissues, This may also be due to the capacity of fish to restore protein and because of additive supplements which might have given strength, support and protection against toxic stress to fish in environment.

**Glycogen Levels**

Carbohydrates are the first substrates to be utilized in metabolism more so under toxic stress conditions. Glucose as the primary fuel is utilized during biological oxidations for energy production. Glycogen and other polysaccharides are broken down by glycolysis into glucose or any other intermediates. The present study provides the evidence that like other organochlorine, carbamate, organophosphorus and synthetic pyrethroids, biopesticides also effect carbohydrate metabolism in different tissues by altering the levels of metabolites and their associated enzyme levels in *Anabas testudineus*. The glycogen content decreased with the increase in sub-lethal concentrations of latex and plant extract of *Calotropis gigantea* in gills, liver and brain, this decrease suggested greater mobilization of glycogen from tissues.
as to meet the toxic stress. The decrease in glycogen levels was in the order of liver, gill and brain. Latex treated fishes were rather more affected than plant extract treated fishes. Fish being subjected to a situation of stress, both catecholamines (Nakano and Tomlison, 1967) and adrenocorticosteroids (Fagerland, 1967; Wedemeyer, 1969) were secreted in increased amounts causing marked changes in carbohydrate reserves in fish (Stimpson, 1965; Nakano and Tomlison, 1967; Swallow and Flemming, 1970; Larson, 1973). These catacholamines increase the process of glycogenolysis leading to decrease in glycogen content which helps to meet the energy demand caused by the toxic environment (Wasserman et al., 1970). Liver is the largest vital organ in the body which mainly stores glucose in the form of glycogen and has function of detoxifying xenobiotic substances. The decrease in glycogen content may be due to increased glycogenolysis and also due to decreased glycogen synthesis. There are reports to show that organochlorine insecticides depressed the activity of glycogen and the activity of glycogen synthetase (Hickenbottom and Yau, 1974). Increase in glycogen phosphorylase activity in liver and muscle was reported by Koundinya and Ramamurthi (1979) and depletion of hepatic glycogen content in Tilapia mossambica exposed to sumithion was also observed. The decrease in glycogen content of all the tissues was observed due to Malathion and Nuvan exposure in Labeo rohitai (Anuradha, 1993). In muscle the depletion is next to brain, this may be due to increased muscular activity as fish tries to move fast after exposing it to pesticides. Depletion in glycogen gill tissue might be due to increased respiratory rate which requires more energy supply to the tissues. Shaffi (1979) reported damage to the surface cells and blood capillaries of gill filaments extensively when fresh water fishes were exposed to heptachlor. A similar fall in glycogen content was observed in the tissues of T. mossambica exposed to Methyl parathion, Malathion and Lindane (Sivaprasada Rao and Ramana Rao, 1979; Kabir Ahmed et al., 1983; Vasanthi, 1983) Depletion of glycogen was also reported in C. Punctatus when exposed to Malathion, Similar reports were available to show the depletion of glycogen in fish, snail and fresh water muscle due to
insecticide toxic stress (Koundinya and Ramamurthy 1979; Srinivasa Murthy et al., 1983). Reduction in the glycogen was also observed in tissues of *S. mossambicus* when exposed to DDT, Malathion and mercury (Ramalingam, 1988). The decrease in glycogen might be due to hypoxic condition which increases carbohydrate consumption. In latex plus supplements treated fishes, the difference in values were less significant. However, certain amount of recovery could be seen. It was interesting to note that, there was a tendency of these values to come nearer to control values, this may also is due to the capacity of fish to restore glycogen and because of additive supplements which might have given strength, support and protection against toxic stress to fish in environment.

**Acid and Alkaline Phosphatase Activity**

Acid and Alkaline phosphates also serve as diagnostic tool to assess the toxicity stress of chemicals in the living organisms (Harper, 1991). Acid phosphatases and Alkaline phosphatases are hydrolytic lysosomal enzymes and are released by the lysosomes for the hydrolysis of foreign material. Hence, they play a role in certain detoxification functions. It is known as inducible enzymes, whose activity in animal tissue goes up when there is a toxic impact and the enzyme begins to counter act. Subsequently the enzyme activity may begin to drop either as a result of having partly or fully encountered the toxin or as a result of cell damage. In the present study on acid and alkaline phosphatase activity, were observed in gills, liver and brain. Similar findings have been reported with other pesticide. Keshavan and Kamble (1984). Lysosomal hydrolases are thought to contribute to the degradation of damaged cells and hence to facilitate their replacement by normal tissue (DeDuve, 1963). Acid phosphatase is an enzyme of lysosomal origin, which hydrolysis the phosphorous esters in acidic medium moreover help in autolysis of the cell after its death. The impact was the highest on the fishes exposed to the highest of the three sublethal concentrations of latex and plant extract of *Calotropis gigantea*. The increase in acid and alkaline phosphatase activity was in the order of liver, gill and
brain. Latex treated fishes were rather more affected than plant extract treated fishes. The fluctuation and increase of acid phosphatase activity encountered in the three sublethal concentrations of latex and plant extract of *Calotropis gigantea* corresponds to the observation made by Singh and Singh (2005); Usha Kaisabhatjao and Vibhuti Rai (1993). Sabita Borah and Yadav (1996) reported increased in acid and alkaline phosphatase activity in gills exposed to pesticide rogor in *Heteropneustes fossilis*. Similar observations were reported by Josephine Paulina (2003) in the sub-lethal concentrations of latex of *Calotropis gigantea* and in *Anabas testudineus* exposed to monocrotophos (Santha Kumar, 1998). The enhanced acid phosphatase might be due to the increase in active uptake of ions through gills. Mullainathan (1982) reported that gill form a major site of accumulation of foreign substances. The higher activity of the acid phosphatase might have influenced the changes in the energy supply of metabolites and it was associated with carbohydrate metabolism. Swamy et al., (1992a) reported increased acid and alkaline phosphatase activity in rat brains, exposed to monocrotophos. Alkaline phosphatase is brush border enzyme that splits various phospho esters at alkaline pH and mediates membrane transport (Goldfisher et al., 1964) and involved in transphosphorylation reactions (Srinivasulu Reddy et al., 1991). Alkaline phosphatase has also been shown to be involved in active transport (Danielli, 1972), glycogen metabolism (Reddy and Rao, 1988), protein synthesis (Pilo et al., 1972), secretory activity (Ibrahim et al., 1974) and in synthesis of certain enzymes (Summer, 1965). Any change in the alkaline phosphatase activity will affect the physiological and biochemical pathways of animals. Increased alkaline phosphatase activity may be indicative of an adaptive rise in enzyme activity to the persistent stress (Murphy and Porter, 1966) as observed in gills, liver and brain. Acid and alkaline phosphatase present in nucleolus are reported to be involved in the synthesis of nucleic acid (Cox and Griffin, 1965) and thus any change in the activities of these enzymes also disturbs the protein synthesis (Srinivasulu Reddy et al., 1991). The behavior of phosphatase activity observed in gills, liver and brain in the present study may be due to the toxic effect of latex and plant extract of
*Calotropis gigantea*, by which the cellular membranes and lysosomal membrane might have been ruptured (*Singh and Singh*, 2005; *Dalela et al.*, 1978) or due to tissue inflammatory reaction of toxin, Increased transphosphorylation activity of the tissue (*Sastry and Sharma*, 1978) may also be a factor, elevation in both phosphatase enzyme activity was observed in gills, liver and brain. Alkaline phosphatase contains a serine residue at its active site (*Mahendra and Agarwal*, 1983) and organo phosphorous insecticides are reported to the potent inhibitors of serine containing enzymes. *Bell et al.*, (1970) and *Rama Rao et al.* (1996) reported increase in activity of alkaline phosphatase in the liver in fish, *Sartherodon mossambicus* exposed to sublethal concentration of carbon tetrachloride. The phosphatases can be consider as metabolic scavengers which break down the non-functional biomolecules to their respective monomeric units to be utilised for other physiological and metabolic functions (*Rama Rao et al.*, 1996). The increased activity of alkaline phosphatase indicated increased cleavage of high energy bonds. The phosphate system comes into operation when the tissue is facing energy crisis (*Rama Rao et al.*, 1996). *Gill et al.*, (1990). It was reported increased in acid and alkaline phosphatase activity in fish, *Clarias batrachus* exposed to acephate. *Ahmad Figar et al.*, (1995) reported increased hepatic acid phosphate increase after two weeks and decreased at the end of four weeks in fish, Chinese grass carp exposed to Danitol. The most probable reason for the decrease in the activity of acid phosphatase observed in liver, in present study could be uncoupling of oxidative phosphorylation (*Dalela et al.*, 1980; *Verma et al.*, 1980). *Simon* (1953) stated that concentrations higher than those needed to prevent oxidative phosphorylation could injure mitochondrial system so as to block the action of enzymes concerned with oxidative metabolism. Action of uncoupling of oxidative phosphorylation has been pointed out on the basis of chemical (*Pressman*, 1963) and chemiosmotic (*Mitchel*, 1961) interactions. According to *Pressman* (1963) uncoupling promote the conductivity of protons within mitochondrial membranes and subsequently prevent a gradient across the membrane. *Weinbach and Garbus* (1969) suggested that
uncoupling traverse through lipoprotein layer of mitochondrial membrane and interact with protein groups that then undergo structural changes. Further, the uncouplers bind tightly with mitochondrial proteins that are involved in aminoacid metabolism. It may be suggested that alteration in membrane permeability, disruption of normal functioning of cell organelles like lysosome and mitochondria, and different repressor mechanisms associated with pesticide toxicity together resulted in significant changes in the level of the enzyme acid phosphatase in the tissues examined. In latex plus supplements treated fishes, the difference in values were less significant. However, certain amount of recovery could be seen. It was interesting to note that, there was a tendency of these values to come nearer to control values, this may also be due to the capacity of fish to restore phosphatase activity and because of additive supplements which might have given strength, support and prevention against toxic stress to fish in environment. The changes observed in the present study with respect to acid and alkaline phosphatase activities in different organs, are due to the toxic effect of latex and plant extract of *Calotropis gigantea*.

**Acetyl cholinesterase Activity**

The most important toxic property of pesticide compounds is inhibiting their target enzyme Acetyl cholinesterase activity (*O' Brein, 1967; Corbeit, 1974*). Most of the organophosphate compounds are similar with the ester part of acetylcholine and they react with esterase part of AchE after entering into the exposed animal .The conversion of acetylcholine into acetic acid and choline catalyzed by AchE is considered to be the key reaction in synaptic transmission (*Bachelard 1976*).

A significant decrease in acetyl cholinesterase activity was observed in the present study during short term exposure. It was recorded in the selected tissues. The decrease in AchE activity was in the order of brain, gill and liver. Latex treated fishes were rather more effected than plant extract treated fishes. The serine hydroxyl group is blocked by op insecticides leading to the inhibition of AchE which may
result in the excessive accumulation of Ach at the synapse, disrupting the transfer of nerve impulse. Similar decrease in AchE activity and increased accumulation of Ach was observed when treated with Roger and Dimecron in fresh water muscle, *L. Marginalis* (*Vijayaendrababu and Vasudev, 1984*). Similar observations were made in fresh water crab, *O. Senex senex* by Fenitrothion (*Bhagyalakshmi and Ramamurthi, 1980*) and in snails exposed to different organophosphorus compounds (*Ramana Rao and Ramurthi, 1979; Anuradha, 1993; Singh and Singh, 2005; Singh and Agarwal, 1982*). The accumulated Ach may induce the synthesis of increased amounts of AchE leading to the revival of affected fishes (*Kabir Ahmed et al., 1980*). AchE was inhibited in both short term and long term. Exposure to Nuvan has resulted in significant changes only during the short term and insignificant during the long term. It was well documented that highly purified phosphorothionates (P=S form) are not direct ‘inhibitors of cholinesterase’ but when they are metabolized to their corresponding oxygen analogues (P=O form) become highly potent inhibitors (*March et al., 1956* and *Murphy et al., 1968*), the susceptibility of animals to poisoning by organophosphorus insecticides will be dependent, at least in one art upon the rate at which the analogues are made available to inhibit cholinesterase at critical site in nerve tissue or organs innervated by cholinergic nerves. Pesticides which inhibit normal function of AchE are known as “Anticholinesterases” agents. With regard to nature of inhibition some of the pesticides are characterized by the nature of inhibition for eg. Carbamate compounds are “Reversible inhibitors” of AchE, as they are less potent and shorter acting anti cholinesterase agents, in contrast OP compounds are known to be “irreversible inhibitors” because of long acting cholinesterase agents (*Koelle, 1975*). Based on the inhibitory activity of AchE, the results indicated that latex was relatively more effective than plant extract. And in latex plus supplements treated fishes, the difference in values were less significant. However, certain amount of recovery could be seen. It was interesting to note that, there was a tendency of these values to come nearer to control values, this may also is due to the capacity of fish to restore AchE activity and because of additive
supplements which might have given strength, support and protection against toxic stress to fish in environment

**Adenosine Triphosphatase Activity:**

**Mg**\(^{++}\) and **Na**\(^{+}\)** K\(^{+}\) ATPase Activity

ATPase activity in general speaks about the transport of Na\(^{+}\) and K\(^{+}\) ions and as well synthesis of ATP. Na\(^{+}\),K\(^{+}\) ATPase is responsible for active cation transport (Skou, 1957). Generally most of the animals maintain high intracellular K\(^{+}\) concentration and a low Na\(^{+}\) concentration and transport of these ions across the cell membrane against an electro chemical gradient takes place by an active transport process. The active transport involves Mg\(^{++}\) dependent, Na\(^{+}\) and K\(^{+}\) activated ATPase which provides the largest contribution to the maintenance of these ionic Transmembrane gradients (Trachtenberg et al., 1981).K\(^{+}\) ATPase is an intrinsic membrane bound protein which hydrolyses ATP to ADP releasing an inorganic phosphate. In this process energy requirement is more (Yates, 1980; Schurmans Stekhovan and Bonting, 1981; Towle, 1981). An important energy producing enzyme in mitochondria is the Mg\(^{++}\) ATPase. Which is shown to be involved in the coupling of ADP + Pi in the biosynthesis of ATP (Boyer et al., 1977). In the present study Na\(^{+}\), K\(^{+}\) ATPase and Mg\(^{++}\) ATPase were estimated. All the three above stated enzyme activities were seen to be reduced during 96 hr exposure. The impact was the highest on the fishes exposed to the highest of the three sublethal concentrations of latex and plant extract of *Calotropis gigantea*. The decrease in Na\(^{+}\), K\(^{+}\) ATPase and Mg\(^{++}\) ATPase activity was in the order of liver, gill and brain. Latex treated fishes were rather more effected than plant extract treated fishes. Among the percentage obtained liver has the maximum amount of decrease followed by gill and brain. The values obtained(Table:13) were statistically significant. These results clearly showed the disturbances in the enzyme activity due to the exposure of the fish to latex and plant extract. In latex plus supplements treated fishes, the difference in values were less
significant. However, certain amount of recovery could be seen. It was interesting to note that, there was a tendency of these values to come nearer to control values. This may also be due to the capacity of fish to restore Na⁺, K⁺ ATPase and Mg²⁺ ATPase activity and because of additive supplements which might have given strength and support against toxic stress to fish in environment. Relatively high concentration of Na⁺ and K⁺ ATPase were recorded in Brain of latex and plant extract treated fish. The inhibition of this enzyme in brain indicates its neurotoxic action and their interference with membrane ionic conductance. There are certain reports from literature that these symptoms can be caused by the inhibition of Na⁺, K⁺ ATPase similar symptoms were observed when rats were injected intracranially with aubain (Bignani and Palladini, 1966) in fish (Anuradha, 1993) the well known inhibitor of active Na⁺, K⁺ transport. In liver due to the impairment of ATPase enzyme activity, there was greater reduction in intracellular metabolism, resulting from the greater reduction of solute transport (Gonzalezcalivin et al., 1983). Considerable inhibition of ATPase in gill, suggested the reduction in osmoregulatory mechanism. Gill epithelium and tight junctions which are supposed to function as channels for salt excretion and osmoregulation might be affected by the toxicants. For uptake of pollutants, gills play primary role and it is the first organ to exhibit symptoms of sub-lethal toxicity. Towle (1981) concluded that Na⁺⁺, K⁺ ATPase plays central role in whole body ionic regulation. Thus any toxicant which interferes with ionic homeostasis will also alter Na⁺⁺, K⁺ ATPase and was reported by many authors, after exposure of a fresh water teleost, Channa gachua to sub lethal level of endosulfan; gill Na⁺, K⁺ ATPase activity was reduced by 20-50% (Dalela et al., 1978) . Mg²⁺ ATPase is associated with oxidative phosphorylation and synthesis of ATP (Desaiah et al., 1979). Considerable decrease of Mg²⁺ ATPase activity was observed after 96 hrs exposure to latex and plant extract. This might be due to the disturbance in the transport of ions through mitochondrial membrane, resulting in a marked effect on the coupling of oxidation to phosphorylation (Ulrich, 1963) the decrease in Mg²⁺ ATPase also indicated decreased ATP synthesis. It was generalized that both in vivo
and in vitro studies showed that the pesticides whether organophosphates, organochlorides or biocides altered the activity of ATPases. In Vitro inhibition of mitochondrial Mg$^{2+}$ ATPase and Na$^+$, K$^+$ ATPase in the brain tissue of blue gill fish under the influence of organochlorines was reported by Cutkomp et al., (1971). Significant inhibition of Mg$^{2+}$ and Na$^+$, K$^+$ ATPase was observed in the brain and gill tissues of Juvenile Mugil cephalus under the influence of three sublethal concentrations of Lindane (Anuradha, 1993). Although the exact mechanism of action is not clearly understood, the inhibition of ATPase suggests that membrane bound enzymes and receptor activities may be partly involved in latex and plant extract exposed fishes. Membrane bound ATPase system is reported to be the prime target for the action of various xenobiotics (Desaiah, 1981; Cutkomp et al., 1982; Desaiah, 1982; Bansal and Desaiah, 1982; Anuradha, 1993). Decrease in Mg$^{2+}$ ATPase in 96hr exposure clearly indicated that the synthesis of ATP was affected in a pronounced way. From these studies it appeared that the decrease in ATPase activity is pronounced, however, certain amount of recovery could be seen in latex plus supplements treated fish.

HISTOPATHOLOGY

The pesticide present in water reaches the fish body through water taken in with food, mucosa of the mouth or gills, they may reach liver, brain, kidney through blood circulation. The percentage of pesticide poisoning was high in the brain. This alters the physiological and behavioural functions of the fish. Various regions in fish brain are concerned with many functions. The impairment of tissues of a region may lead into the curtailment of the particular function. This has become proved from the classical work of Ariens Kappers et al., (1963) relating the functions of brain in various fishes. It is described that, the gross morphology of the brain of some Indian fishes and correlated its structure with the feeding habit and habitat of fishes. Ito (1970) discussed the functional significance of the carp optic tectum. On the basis of the various experimental evidences, it has been established that brain is the
controlling centre for all functions and movements in the body serving as a relay station. Signs of brain toxicity due to exposure to Fenvalarate in fish include tremors which progress to convulsion (Bradbury et al., 1987). The effect of latex and plant extract on brain showed vascular dilation that is corresponding to earlier observation by Cope et al., (1970) and Sajitha Bhaskar (1994). The rupture of the wall of brain found in the present works coincides with the work done by Kennedy et al., (1970) in methoxychlor and Di Michele and Taylor (1978) on naphthalene. Aoki (1978) on heavy metals, Gardner and La Roche (1973) on Copper chloride, Reichenback Klinke (1975) on chemotherapeutic agents, Josephine Paulina, 2003; Preetha Kumari, 2004; Bharathi (2005) in Anabas testudineus with latex and biopesticide of Calotropis gigantea. Santha Kumar (1998) with monocrotophos in Anabas testudineus were consistent with the present observation (plate 1 and 2). The pathological examinations, of intracellular space and irregularly shaped nuclear arrangement coincides with the work done on industrial effluent by Joshi and Dubey (1984). However, the fish exposed to latex plus supplements showed less damage to the tissue due to the presence of additive nutrients, which might act as antioxidants (savior against free radical induced oxidative damage) enhancing proper coordination and thus, protects from fish mortality.

Histological observation on gills showed that latex and plant extract induced fish showed remarkable pathological changes after 96hr exposure. In fish, the gill is the most important organ for respiration and osmoregulation and it is the first organ to which the pollutant comes into contact. Hence, it is more vulnerable to damage than any other tissue. The pathological conditions include separation of epithelial layer over secondary gill filaments, necrosis in inter lamellar spaces and fusion of secondary gill lamellae (Plate: 3 and 4), swelling of secondary gill filaments and bulging of primary gill lamellae. Necrosis is in the central axis region and atrophy or secondary gill filaments. Some of these changes were noticed in fishes under exposure to different pesticides by Vijayalakshmi and Tilak (1996); Moses Girija
and Jayantha Rao (1995); Chakrabarthy and Konar (1974). In the fish, malathion has been shown to induce histopathological changes in gills (Singh and Sahai, 1984). Josephine Paulina, 2003; Preetha Kumari, 2004 with latex and Bharathi (2005) in *Anabas testudineus* with biopesticide of *Calotropis gigantea*. Santha Kumar (1998) with monocrotophos in *Anabas testudineus* were consistent with the present observation. Hyperplasia of primary filaments and secondary lamellae were reported in the gills *Puntius* exposed to Khan River water with industrial sewage (Chouhan and Pandey, 1987). Hyperplasia of branchial epithelium was found to be the most pronounced damage caused to *Anabas testudineus* because of toxicants. Osburn (1910) has concluded that proliferation of gill epithelium protects the gill filaments from constant irritation in silver salmon fingerlings that lack adequate gill covers. But, since proliferative thickening of gill epithelium produced by most kind’s environmental toxicity (Skidmore and Tovell, 1972; Roberts, 1978). This response considered as general safety measure against irrigation by environmental toxicants. Hyperplasia of branchial epithelium has been observed commonly in fish facing low tension of oxygen and high concentrations of wastes in the environment. (Roberts, 1978). Lifting of lamellae epithelium usually occurs when the lymphoid space between the epithelium and its supporting elements gets enlarged by accumulation of fluid on account of factors like increased capillary permeability (Roberts, 1978) or lowered efficiency of the epithelial cells in maintaining normal water balance (Skidmore and Tovell, 1972). Inflammatory alterations of lamellar epithelium and hyperplasia were reported in the gills of fresh water major carp *Cirrhinus mrigala* (Hamilton) during 48 hr exposure to sublethal dose of Malathion (Roy and Datta Munshi, 1991). Oedema with lifting lamellar epithelium and hyperplasia of epithelium was observed in the gills of all cat fish containing residues of Endosulfan (Nowk and Barbara, 1992). The changes that took place in the gill of latex and plant extract exposed fish result from the direct contact with the organ. In latex and plant extract of *Calotropis gigantea* exposed fish bulging of tip of primary gill filaments, curling of secondary gill filaments, necrosis in the
cells and development vacuoles are observed. Similar observations were found in the gills of *Puntius aurilus* (*Bengeri Patti, 1987*) and in *Tilapia mossambica* exposed to malathion an organophosphorous insecticide (*Rao et al., 1983*). In fish, the respiratory epithelium is the barrier between the blood and the surrounding water through which respiratory gases and other materials needed for sustenance are exchanged. Any damage to this epithelium impairs not only the ventilator process but also other vital processes, like ion-exchange, during the secretory and excretory functions of the gills. Fusion of gill lamellae and lifting of gill epithelium from the supporting elements are such effect which reduce the surface area available for gaseous and other exchange and lengthen the distance over which the exchange diffusion occurs (*Jargoe and Haines, 1983*); ultra structural features like loss of surface macroridges of secondary lamellar epithelial cells also produce similar reduction in the exchange surface (*Jagoe and Haines, 1983*). When this hampers the exchange of respiratory gases across the gill epithelium then respiratory insufficiency becomes a natural consequence. Respiratory distress also results when stress-induced damage in the gill epithelium lead to events like increased influx of hydrogen ions that reduce the pH of the blood and thus decrease the oxygen carrying capacity of haemoglobin (*Haines and Schofield, 1980*). Study on hematological parameters has shown the oxygen combining capacity of the blood was reduced in fishes exposed to latex and plant extract. On the other hand, the ion regulatory and excretory functions of the gill are hampered when epithelial damage disturbs the exchange of ammonium and bicarbonate ions of the blood with sodium and chloride ions of the medium that normally occurs across the gill epithelium of fish (*Love, 1980*). Mucus cells in the secondary lamellae of the experimental fish are more than in control fish, indicating the responses of the fish to the toxicant. Effects like collapse of pillar cell system and rupture of gill epithelium tend to stagnate or even stop the lamellar blood flow (*Skimore and Tovell, 1972*). This consequence is also likely to limit the respiratory capacity of the gills. Degeneration of epithelial cells indicates the damage in the gill lamellae that reduces the activity. Undoubtedly, therefore, as stipulated by *Eller*
(1975), gill alterations, such as those observed presently, would represent basic physiological problems that the fish under stress may not ultimately be able to cope with. The fish exposed to latex plus supplements showed less damage to the tissue and the presence of additive nutrients might have protected from damage of gill epithelium increasing respiratory capacity of gills of fish preventing distress and mortality.

The liver showed marked cytoplasmic granularity, periportal atrophy and radial disorientation. These conditions are similar to the cirrhosis (Boyd, 1949) and earlier observations of Eller (1971) and Verma et al., (1975). The loss in the tubulosinusoidal arrangement in the present study is in the liver of L. Macrophirus treated with heptachlor also corresponds to the findings of Eller (1971) in S. Clarki treated with 10 ppb endrin for 5 months. Hyperophy of the hepatic cells as reported by Mathur (1965) Sastry et al., (1977) is noted in the present study. There was increased cytoplasmic granularity and might be due to the loss of vacuolation and shrinkage of hepatic cells as reported by Kennedy et al., (1970) in L. Macrophinus exposed to methoxychlor. Pyconsis of nuclei was previously described by Cope et al., (1969) in dichlofen exposed in blue gills and Bhattacharya et al., (1975) in endrin treated Channa punctatus while in DDT exposed trout (King, 1962), endosulfan treated Anabas testudineus (Satheesh Kumar Reddy, 1994); lindane treated Anabas testudineus (Zayapragasaarazan, 1993); tri-aromatic hydrocarbon treated cat fish, Heteropneustes fossilis (Dwivedi and Rajakamal Sarin, 1996) and latex treated Tilapia mossambica (Desai et al., 1984). And in the histological and cytochemical indices that lysosomal perturbations in dab Limanda limanda indicated early tissue lesions. Impairment of the lysosomal membrane stability, pathological lipid accumulation and Liver histopathology was also reported by them along the North sea. The early fibrosis as reported by Cope et al., (1970) in blue gills also noticed in the present study. The deformations of hepatocytes as observed in endrin treated cut throat trout (Eller, 1971), Clarias batrachus treated with alloxan (Goel and Agrawal,
1977) and Mystus tengara exposed to carbay and endrin (Toor et al., 1977) is one of the observation of this study. The development of intercellular spaces and large gaps (Plate: 5 and 6) in the parenchymal mass might be the factors of disorientation of liver cords. Congestion of blood might be the factors for disorientation of liver cords. Congestion of blood in the central vein and partial loss of radial orientation, parenchymal shrinkage and increased cytoplasmic granularity are consistent with hepatitis described by Boyd (1949). The results in the present study coincide with observations made by Santha kumar (1998) with monocrotophos , Josephine Paulina, 2003; PreethaKkumari, 2004; Bharathi, 2005 in Anabas testudineus with latex and biopesticide of Calotropis gigantea in Anabas testudineus (Plate: 5 and 6). Walsh and Ribelin (1975) in fishes on exposure to endosulfan and other pesticide reports. King (1962) reported necrosis of the tubular epithelium that coincides in the present observations. The interstitial cells were found as foamy masses with deeply stained nuclei. Similar observation was also reported by Areechon and Plumb (1990) in Channel catfish, Ictalurus punctatus exposed to malathion. Results in this work showed severe necrosis, pyknosis and disintegration of hepatocytes, were very much evident in this test level. These responses can cause severe physico-metabolic dysfunction leading to death. Fish mortalities observed in such situation may then be related to complete hepatocyte degeneration, Hemorrhage of blood vessels followed by clumping and scattering of erythrocytes were also seen. However, in the present studies, the fish exposed to latex plus supplements showed less damage to the tissue. This may be due to the presence of immuno-modulators and additive nutrients, which might have prevented from hepatic abnormalities and disturbance in liver functions, promoting normalacy, protecting fish from mortality.